Implementation of new concentration technologies for microbiological recoveries in a drinking water system from Aquavalens project

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Abstract

A new concentration method for microbiological analysis has been implemented in a routine sampling and event or emergency situation in a Drinking Water System (DWS), including Drinking Water Treatment Plant (DWTP) and Distribution Network (DN). This concentration method has been developed during European Aquavalens Project (an EU funded -7th Framework Programme- that aims to protect the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation). This new protocol is based on a hemodialyzer membrane filter, able to concentrate high water volumes and recover three different kingdoms (viruses, bacteria and protozoa). Field samples were taken from DWTP and DN on a monthly period during one year. Parametres such as E. coli, Campylobacter spp, Legionella spp and Legionella pneumophila, Norovirus GI/GII, Hepatitis A virus, Giardia spp and Cryptosporidium were tested by using molecular methods (qPCR and FISH). Obtained results from these new methodologies were compared to standarized and/or validated methods, showing better sensibility and recoveries in most cases. Data is used to calculate treatment efficiency at DWTP (logarithmic reduction of the microbiological load at different treatment steps), to assess DN quality water through Water Safety Plans (WPS) and improving Standard Operational Protocols (SOPs). It is also a less time- consuming (sampling and analyzing) method, saving money and human resources. Acknowledgments: This study has been funded by the European Union through the project AQUAVALENS.

Keywords:

Microbiological recoveries in a drinking water system

Recovery of target microorganisms:

Traditional approaches to the isolation of microbial indicators have relied on various agar plate and liquid media methods. The basic pour plate technique has a maximum sample volume of about 1 ml whereas the spread plate technique uses 0.1 or 0.2 ml samples. For larger volume processing and rapid throughput, however, the membrane filtration technique is preferred if interfering particles are not concentrated simultaneously. Liquid cultivation techniques, either for the detection of the target organism (presence/absence test) or quantitatively, using multiple tube techniques and most probable number (MPN) calculations, allow flexible sample volume range and the handling of turbid samples. In liquid cultivation techniques, small volumes of sample dilutions or up to ten litre samples can be used. The detection of target microorganisms by non-cultivation methods is also presented for enteric viruses and parasitic protozoa.

Filtration methods:

Bacteria are generally recovered on 47 mm diameter membrane filters with porosities of 0.22 to 0.45 µm. Membrane filters may be incubated on solid media, pads soaked in liquid media or as a MPN system in enrichment broth. Cysts of protozoan parasites can be recovered on similar membranes but with larger surfaces (up to 293 mm diameter) and porosities as high as 2 µm (Ongerth and Stibbs, 1987). For convenience, however, various cartridge filters 239 are generally preferred to recover protozoan cysts from up to 100 l water samples even in the presence of some turbidity (USEPA, 1999). The coconcentration of non-target particulates can, in part, be removed by subsequent selective separation method(s) (such as immunomagnetic separation (IMS), gradient centrifugation or flow cytometry.

Detection, identification and quantification of microorganisms:

This section describes the more “classical” methods, which depend largely on cultivation techniques, as well as molecular methods. A number of them, particularly most of the recent techniques require standardisation and validation. Nonetheless, the majority of the methods presented here have already proven to be useful in drinking water microbiology and/or medical diagnostics, or display great potential. In the detection, identification and quantification of target organisms some approaches are solely based on a single technique whereas other strategies take advantage of a combination of different methods. For example, to identify Escherichia coli reliance can be placed on a one-day-cultivation on chromogenic

media. Alternatively, in a much faster approach, short precultivation on an artificial medium can be combined with labelling using fluorescent probes, microscopy, and laser scanning techniques . In the following sub-sections, alternative approaches are offered for a number of target organisms. The traditional cultivation techniques are usually sensitive but the identification is often not as reliable as might be desired. Methods based on molecular biology tend to be sensitive and yield reliable identification, but cultivation techniques always show viable organisms whereas molecular methods often reveal dead or inactivated target organisms/nucleic acid. This is of relevance in disinfected waters and should be considered in the interpretation of results.